WT-MscS and the alanine mutants have been undertaken to experimentally test the predictions from the modelling studies.

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2268-Plat

Novel Mutations in the Extracellular Cap of the Mammalian Mechanosensitive Channel TREK-1

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The Twik related potassium channel 1 (TREK-1) is one of the best studied mechanosensitive mammalian channels. TREK-1 is known to play very important roles in depression, ischemia and vasoregulation. TREK-1 belongs to the family of background or "leak" potassium channels are constitutively open at rest and have a central role in the tuning of neuronal resting membrane potential, duration of action potential and regulation of neurotransmitter release. These K^+ channels are members of the family of K2P channels subunits containing four transmembrane and two pore domains. The functional channel is formed by two subunits and is predicted to have a two-fold symmetry.

Here we show the feasibility of the use of microbial genetics to study the structure-function relationship of this mammalian channel. The advantage of this approach is that we can directly screen for channels with altered phenotypes and correlate this altered function with structural changes. We have successfully expressed a functional TREK-1 channel in bacterial cells, and show that it can partially rescue the slow growth phenotype of an E .coli strain deficient in three mayor potassium transporters. Furthermore, using random mutagenesis and bacterial screens we have isolated five mutants that better remediate the potassium deficiency of this bacterial strain. Because these mutants clustered in a stretch of 20 amino acids in the extracellular cap of the TREK-1, we think that we have found a functional "hot spot" by utilizing the power of bacterial genetics.

2269-Plat

Omega 6 Polyunsaturated Fatty Acid-Containing Phospholipids Enhance Neuronal Cell Mechanics and Touch in C. Elegans

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Mechano-electrical transduction (MeT) channels embedded in neuronal cell membranes are essential for touch and proprioception. Little is understood about the interplay between native MeT channels and membrane phospholipids, in part because few techniques are available for altering plasma membrane composition in vivo. Here, we leverage genetic dissection, chemical complementation, and optogenetics to establish that arachidonic acid (AA), an omega 6 polyunsaturated fatty acid (PUFA), enhances touch sensation and mechanoelectrical transduction activity while incorporated into membrane phospholipids in C. elegans touch receptor neurons (TRNs). We found that arachidonic acid acts cell autonomously, since we show that enzymes needed for its synthesis are expressed in TRNs. We also established that the membrane viscoelastic properties of TRNs lacking omega 6 PUFAs are altered (i.e., membrane bending and viscosity), yielding less flexible membranes than wild type, as determined by atomic force microscopy (AFM) based single-tether extrusion. Our data suggest that the defect in touch sensation likely reflects a loss of mechanotransduction rather than lack of excitability or downstream signaling. These findings establish that polyunsaturated phospholipids are crucial determinants of both the biochemistry and mechanics of mechanoreceptor neurons and reinforce the idea that sensory mechanotransduction in animals relies on a cellular machine composed of both proteins and membrane lipids.

2270-Plat

Touch Activates Mechanosensitive Ion Channels in Merkel Cells In Vitro

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Merkel cell-neurite complexes are gentle touch receptors that mediate slowly adapting type I (SAI) responses. Since their description in 1875, Merkel cells have been proposed to be mechanosensory cells that transduce mechanical stimuli into electrical signals that activate somatosensory neurons. Consistent with this model, conditional knockout mice that lack Merkel cells show the loss of touch-evoked SAI responses. Moreover, in vitro studies on cultured Merkel cells report calcium elevation in Merkel cells in response to swelling or membrane stretch. Previous studies support the contribution of Merkel cells to touch sensation, however, the central question of whether Merkel cells are intrinsically touch sensitive is unanswered. To tackle this problem, we performed live-cell imaging and electrophysiological recordings from mouse Merkel cells. Merkel cells were dissociated from the epidermal skin of transgenic Atoh1/nGFP mice, whose Merkel cells selectively express green fluorescent protein. GFP-positive cells were purified using fluorescence-activated cell sorting and cultured for 1-5 days. Individual Merkel cells were stimulated by families of displacements (≦0.3-μm steps) with a glass probe driven by a piezoelectric actuator. Touch-evoked responses were monitored with either ratiometric calcium imaging or tight-seal, whole-cell recordings. Merkel cells displayed reversible calcium responses to focal displacements applied to somata. Moreover, electrophysiological recordings demonstrated mechanically activated inward currents at a negative holding potential. Peak inward currents ranged from 107.3-431.3 pA. The 10-90% operating range of these mechanically activated currents was 1.7 ± 0.1 μm (N=10 cells, mean ± SE). Like mechanosensitive currents in hair cells and somatosensory neurons, Merkel-cell currents adapted exponentially to sustained stimuli. Quantitative PCR indicated that Merkel cells expressed both Piezo1 and Piezo2 genes. Together, these data demonstrate that Merkel cells are intrinsically mechanosensitive in the absence of other skin cells or somatosensory neurons.

2271-Plat

Molecular Mechanisms of Deafness Mutations Disrupting Tip-Link Function in Hair-Cell Mechanotransduction

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Hair-cell tip links are fine filaments that directly convey mechanical force to inner ear mechanotransduction channels. These filaments are made of protocadherin-15 (PCDH15) and cadherin-23 (CDH23), two deafness-related proteins that feature long extracellular domains interacting tip-to-tip in a calcium dependent manner. Here we combine X-ray crystallography, molecular dynamics simulations, and binding experiments to explore the molecular mechanisms by which deafness mutations disrupt tip-link function in hair-cell mechanotransduction. We find that these mutations disrupt tip links through impaired interaction between PCDH15 and CDH23 (PCDH15-R113G and PCDH15-I108N), impaired calcium binding (CDH23-D1010G), subtle weakening of structural stability (CDH23-S47P), or impaired folding (PCDH15-D157G). Interestingly, the biochemical effects of each of these deafness mutations correlate with the severity of the reported inner-ear phenotype. Our results shed light on the molecular mechanisms of hair-cell sensory transduction and may help develop tailored treatments for cadherin-mediated deafness.

Platform: Force Spectroscopy

2272-Plat

Disulfide Bonds are Allosteric Regulator of Mechanical Stability

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Disulfide bonds are known to stabilize proteins against perturbations such as temperature or denaturants. Since mechanical force is the most common protein denaturant in vivo, there is increasing interest in the role that disulfide bonds have in the mechanical unfolding of proteins. For instance, disulfide bonds reduce the contour length of stretched proteins by limiting the extensibility up to the covalently linked cysteines. However very little is known about the effect of native disulfide bonds in the strength of structural clamps that determine mechanical stability.

Here, we use single molecule atomic force spectroscopy to study the mechanical effects of disulfide bonding in immunoglobulin domains (Ig) that are constitutively under force.

We observe that the formation of native disulfide bonds in immunoglobulin domains triggers drastic differences in the rupture forces, although the two cognate cysteines do not link together the β-strands of the mechanical clamp motif. In the case of bacterial gram-negative pilin FimH, disulfide increases the most probable unfolding force from 297 pN to 425 pN at 400nm/s. In contrast, the disulfide in the 69th immunoglobulin domain of human titin decreases the unfolding force at a pulling rate of 1200nm/s from 271 pN to 195 pN. We observe that both in titin and in the Fim pilus, the disulfide bonds are remarkably conserved along the entire stretched molecular architecture. Hence, many Igs in elastic segment of titin and in all Fim Igs are predicted to change mechanical stability depending of their oxidation state. Our results

suggest that native disulfide bonds alter allosterically the transition state and modulate the internal flexibility of the stressed protein prior the breakage of its mechanical clamp motif.

2273-Plat
Quantifying the Resolution of Single-Molecule Torque Measurements by Allan Variance
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Single-molecule manipulation techniques have provided unprecedented insights into the structure, function, interactions, and mechanical properties of biological macromolecules. While many single-molecule manipulation techniques naturally operate in the space of (linear) extension and force, recently a number of techniques have been developed that enable measurements of rotation angle and torque. Examples include the rotor bead tracking assay, the optical torque wrench (OTW), and magnetic torque tweezers (MTT). While systematic analyses of the position and force resolution of single-molecule techniques have attracted considerable attention (see e.g. [1,2]), detailed analysis of the angle and torque resolution is currently lacking.
Here, we propose Allan variance as a criterion to systematically quantitate the angle and torque resolution in single-molecule measurements. We apply the Allan variance method to experimental data from our implementations of MTT [3,4,5] and an OTW [6]. Both magnetic and optical torque tweezers can achieve a torque resolution of better or equal to 1 pN•nm. We find that our state-of-the-art OTW outperforms MTT for short measurement times. However, for measurement times > 10 s, drift becomes a limiting factor in the OTW and the superior stability of MTT accomplishes higher ultimate torque resolution for long measurement times.
In summary, our Allan variance criterion enables to critically assess the torque resolution as a function of measurement time and across different measurement modalities that rely on distinct physical principles. In addition, the Allan variance method provides a tool to optimize the measurement protocol for a given instrument and system.
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2274-Plat
Combined Single Molecule Force and Fluorescence Spectroscopy of the Unfolding and Refolding of Green Fluorescent Protein
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We have used optical tweezers to study the free energy surface for unfolding-refolding of the green fluorescent protein (EGFP) and simultaneously monitored the loss and recovery of its fluorescence. This conformational landscape shows unfolding intermediates, molten globe refolding intermediates, as well as misfolded states. As force is used to drive transitions between conformational substates, single molecule fluorescence is probed. In its native state, the emission of EGFP is punctuated by transient dark states ("blinking"); this emission is lost and regained as the protein is unfolded and subsequently refolded. These results provide a full understanding of the unfolding-refolding energy landscape of EGFP and how the conformational state affects the environment of the fluorophore, which reconciles three classes of previous experiments: (1) bulk unfolding/refolding with fluorescence (2) single molecule force-unfolding and (3) single molecule fluorescence intensity fluctuations. This investigation is relevant to efforts at developing EGFP as a genetically encoded force sensor, as well as its use in single molecule imaging and fluorescence recovery after photobleaching experiments.

2275-Plat
VWF - Collagen Interactions Studied with Single Molecule Force Spectroscopy
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Von Willebrand factor (VWF) is a huge multimeric protein that plays a key role in hemostasis. Sites for collagen binding, an initial event of hemostasis, are located in domains A1 and A3 of VWF. Collagen III is believed to interact with the A3-domain, and collagen VI with the A1-domain. The forces and the dynamics of these interactions were investigated with molecular recognition force spectroscopy (MRFS), using substrates with a dense layer of poly (ethylene glycol) chains and terminal benzaldehyde functions for covalent immobilization of collagen. The bond between collagen III and the A3-domain of VWF domain construct A1-A2-A3 was more stable than the bond between collagen VI and the A1-domain of A1-A2-A3, suggesting that A3 is the main binding domain for collagen. We also investigated a mutation in the A3-domain of A1-A2-A3 (S1731T) that shows a slight decrease of collagen III binding determined by ELISA. In MRFS interactions between collagen III and the S1731T mutant showed no significant difference in stability compared to the wild type construct. These data are consistent with our observation that persons with mutation S1731T exhibit only a mild or no significant bleeding tendency. We further compared the collagen VI binding capability of A1-A2-A3 and A1-A2. The bond between collagen VI and A1 was stronger when the A3-domain was missing. In addition, the injection of free A2-domains disturbed the collagen VI - A1 interaction, but had no effect on interactions between collagen III and the A3-domain, indicating that domain A1 might also interact with A2. Our data allow deriving a detailed molecular picture on the interplay of collagen-VWF-domain interactions. *This work was supported by the German Research Foundation (DFG Research Unit FOR 1543 - SHENC) and the Austrian Science Fund (Project I 767-B11).*

2276-Plat
Resolving the Molecular Determinants of Cadherin Catch Bond Formation
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Classical cadherin Ca^{2+}-dependent cell-cell adhesion proteins play key roles in embryogenesis and in maintaining tissue integrity. Cadherins mediate robust adhesion by binding in multiple conformations. We recently showed that one of these conformations, called an X-dimer, forms catch bonds that strengthen and become longer lived in the presence of mechanical stress. Here we use single molecule force clamp spectroscopy with an Atomic Force Microscope along with Molecular Dynamics and Steered Molecular Dynamics simulations to identify key interactions that mediate X-dimer catch bond formation and to resolve the role of Ca^{2+} ions in this process. We show that tensile force bends the cadherin extracellular region such that they form force-induced hydrogen bonds that lock X-dimers into tighter contact. When Ca^{2+} concentration is decreased, these hydrogen bonds are eliminated and catch bond formation is abolished. Based on these results, we formulate a simple *'flex and lock'* kinetic scheme that quantitatively describes X-dimer catch bonds.

2277-Plat
Catch Bond Interaction Between Glycosaminoglycans and Cell Surface Sulfatase Sulf1
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In biological adhesion, the biophysical mechanism of specific, non-covalent, biomolecular interaction can be divided in slip- and catch-bonds, respectively. Conceptually, slip bonds exhibit reduced bond lifetime under increased external loads whereas catch-bonds, in contrast, increased lifetime for a certain force interval. Since 2003, a handful of biological systems such as the adhesive proteins P-Selectin and FimH have been identified to display catch-bond properties.
Upon investigating the specific interaction between the unique hydrophilic domain (HD) of human cell-surface sulfatase Sulf1 against the native glycosaminoglycan (GAG) target heparan sulfate (HS) by single-molecule force spectroscopy (SMFS), we found clear evidence of catch-bond behavior in this system. The HD, about 320 amino acids long and strongly positive charged, and the GAG-polymers, composed of up to 200 disaccharide units, were quantitatively investigated with atomic force microscopy (AFM) based dynamic force spectroscopy (DFS) as well as force-clamp spectroscopy (FCS). The observed catch-bond character of HD against GAGs was found to be specifically related to the GAG 6-O-sulfation site. Therefore, this behavior can also be found in HS-related GAGs like heparin and (to a lesser extent) dermatan sulfate, whereas in contrast, only slip-bond binding can be observed in a GAG system where these sites are explicitly lacking. Our observed catch-bond binding data can be interpreted within the theoretical framework of a force mediated transition between two slip-bond regimes. Interestingly, the transition between the two states occurs in a force interval of only 5 Piconewtons while the lifetime of the adhesion bond increases approximately 5-fold for heparan sulfate and heparin.